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Immunology

Partial amino-acid sequences of human and rabbit C-reactive proteins: Homology with immunoglobulins and histocompatibility antigens

(evolution/complement/immunoglobulin domain)

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ABSTRACT **Partial amino-acid sequence analyses of the amino terminus of rabbit C-reactive protein and of a peptide isolated from human C-reactive protein after cyanogen bromide cleavage show an extensive sequence homology between these proteins. Computer analysis detected a distant but significant homology between rabbit C-reactive protein and the C_H3 domain of human IgG. In addition, an examination of the limited data available for the amino-acid sequences of human and mouse histocompatibility antigens revealed a similarity between these proteins and C-reactive protein and, therefore, immunoglobulins. These relationships are presented as evidence in support of the hypothesis that C-reactive protein and immunoglobulins share, in addition to functional similarities, a common evolutionary origin with the major histocompatibility antigens.**

C-reactive protein (CRP) is a distinctive trace constituent of normal serum that is elevated up to 1000-fold during acute inflammatory reactions. The determination of CRP levels has been widely accepted as a useful index of acute inflammation although the biological functions or *raison d'être* of this protein remain obscure. CRP was initially defined by a unique, calcium-dependent precipitation reaction with the pneumococcal C-polysaccharide (1, 2). In addition, CRP is an agglutinin (3) and an opsonin (4), and, in the presence of a suitable ligand, CRP activates the complement system to cause depletion of complement component hemolytic activity (5–7) and promotion of complement-dependent opsonization (8) and hemolysis (9). This array of properties, reminiscent of specific antibody, has prompted us to postulate shared functions and a common evolutionary origin for CRP and immunoglobulins. We have undertaken to determine the primary structure of both rabbit and human CRP in order to test this hypothesis. We report here extensive NH_2-terminal sequence analysis of rabbit CRP and the isolation and characterization of a homologous peptide from human CRP. Several computer-based analyses were performed to search for homology with immunoglobulin. Thus far, significant homology has only been detected between CRP and the C_H3 domain of human IgG1. We also describe an apparent homology between CRP and the recently published sequences of human and murine histocompatibility antigen heavy (H) chains. *Ipso facto,* this has defined a region in the immunoglobulin domain that is homologous to the NH_2-terminal region of these antigens.

MATERIALS AND METHODS

Isolation of C-Reactive Protein. Human CRP was purified from human serous fluids by affinity chromatography and gel filtration (5). The preparations used in this study were pure by immunoelectrophoresis and polyacrylamide gel electrophoresis (9). Rabbit CRP was isolated in a similar manner from acute phase serum of rabbits injected 2–3 days previously with croton oil (1%) in paraffin oil. Rabbit CRP is similar to human CRP in subunit molecular weight (22,900), amino-acid composition, and phosphocholine-binding specificity (10). CRP solutions were exhaustively dialyzed against water and lyophilized.

Cyanogen Bromide Cleavage of Human CRP. Human CRP was dissolved in formic acid, and the concentration of formic acid was adjusted to 70%. A 500-fold molar excess of cyanogen bromide over methionine residues (two methionine residues per CRP subunit) was added and cleavage was allowed to proceed at 22–24° for 18 hr. After reduction with 0.05 M dithiothreitol and alkylation with 0.12 M iodo[^{14}C]acetamide in 6 M guanidinium chloride, the peptides were fractionated in 6 M guanidinium chloride (11) on Bio-Gel A-1.5m.

NH_2-Terminal Sequence Determination. NH_2-terminal amino-acid sequences were determined with a Beckman sequenator (model 890B). The sequence determination was facilitated by a modified dimethyallylamine sequencer program in which 1-chlorobutane serves as both the S_1 and S_3 solvents (12). Double cleavage runs in which both S_3 extractions were collected were used throughout (12). Phenylthiohydantoin amino acids were identified as such or as their trimethylsilyl derivatives by gas chromatography (13). A second method of identification routinely used was hydrolysis with HI (14) followed by amino-acid analysis with a modified (12) Beckman amino-acid analyzer (model 120C).

RESULTS

Sequence Determination. Rabbit CRP contains an unblocked amino terminus and is directly amenable to automated Edman degradation. The partial amino-acid sequence for rabbit CRP deduced from a 51-cycle sequenator run is shown in Fig. 1. Human CRP contains a blocked amino terminus and its sequence could not be determined directly. The presence of 2.0 methionyl residues per subunit, however, enabled the isolation of unblocked fragments after digestion with CNBr. Gel filtration of CNBr digests of reduced and alkylated human CRP revealed two major fragments, designated as CNBrI and CNBrII in order of their elution from the column. The molecular weights of these fragments were estimated by including reduced and alkylated standard proteins on analytical gel filtration columns (15). As shown in Fig. 2, CNBrI and CNBrII behaved as polypeptides with chain lengths of about 150 and 40 residues, respectively.

Amino-acid analyses of CNBrI and CNBrII after conversion of homoserine lactone to homoserine (16) showed that CNBrI

Abbreviations: CRP, C-reactive protein; C and V regions, constant and variable regions; H chain, heavy chain; C1t, subcomponent of complement component C1.

	5					10								15					20					
Rabbit CRP	Ala	Gly	Met	His	Lys	Lys	Ala	Phe	Val	Phe	Pro	Lys	Glu	Ser	Asx	Asx	Ser	Tyr	Val	Ser	Leu	Asx
Human CRP-CNBrI			Met	Ser	Arg	Lys	Ala	Phe	Val	Phe	Pro	Lys	Glu	Ser	Asp	Thr	Ser	Tyr	Val	Ser	Leu	Lys

	25						30						35				40						45					50				
Rabbit CRP	Ala	Gly	Leu	Lys	Lys	Pro	Leu	Lys	Ala	Phe	Thr	Val	Ser	Leu	Tyr	Phe	Tyr	Thr	Asx	Leu	Ser	Met	Thr	Asx	Gly	Tyr	X	Ile	Phe			
Human CRP-CNBrI	Ala	Pro	Leu	Thr	Lys	Pro	Leu	Gly	Ala	Phe	Gly	Val	X	Leu																		

FIG. 1. Partial amino-acid sequences of rabbit CRP and of a fragment, CNBrI, from human CRP. Both preparations had been reduced and alkylated with iodo[^{14}C]acetamide to aid in distinguishing serine from half-cystine. In addition to the major sequence shown for rabbit CRP, valine was found at position 2 and glutamic acid or glutamine at position 37. There was no other evidence of heterogeneity. For the rabbit CRP sequence determination, an average repetitive yield of at least 97% was obtained with an initial yield of over 70%. For the CNBrI fragment of human CRP, the average repetitive yield was 96–97% and the initial yield was about 40%. There was no evidence of a "preview" effect due to the histidine residue in rabbit CRP at residue 4.

contained 1.0 residue, while CNBrII was devoid, of homoserine. Their order in the intact protein must therefore be CNBrI-CNBrII. Both fragments contain unblocked NH_2-terminal groups and thus neither fragment includes the blocked NH_2 terminus of human CRP. A third fragment, CNBrIII, represents the blocked oligopeptide sequence which begins at the NH_2-terminal end of the molecule and ends at the NH_2 terminus of CNBrI. Fragment CNBrIII has not yet been isolated in pure form and was not detected under the conditions used here.

The partial amino-acid sequence of CNBrI is also shown in Fig. 1. A strong and obvious homology with the NH_2-terminal sequence of rabbit CRP was found for the sequence of CNBrI but not for the sequence of CNBrII (Osmand and Friedenson, unpublished data). This was taken as evidence confirming the order of these fragments in the intact CRP subunit.

Computer Analyses of Sequence Data. The sequence data (Fig. 1) were tested for homology with immunoglobulin domains by the method of Fitch (17, 18). The results of this analysis (Fig. 3) when lengths of sequence of 45 residues were examined showed that rabbit CRP has a significant sequence homology (P = 0.008) with the C_H3 domain of human IgG1; significant homology could not be detected between CRP and the C_H1 or C_H2 domains of IgG. The evolutionary distance between CRP and the immunoglobulins is probably large. Thus, when Fitch analysis was used to compare segments of 30 residues, the probability that the homology between CRP and the C_H3 domain of IgG1 occurred by chance rose by an order of magnitude to 0.08. Variation in the length of sequence examined had the reverse effect when rabbit CRP was compared to the F_c fragment of IgM (Fig. 3); significant homology (P =

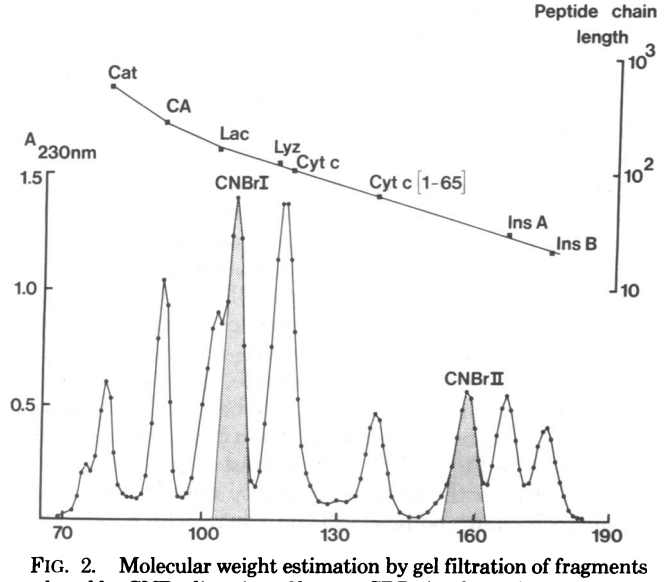

FIG. 2. Molecular weight estimation by gel filtration of fragments produced by CNBr digestion of human CRP. A column (1.5 × 100 cm) of Bio-Gel A-1.5m (200–400 mesh) was used with 6 M guanidinium chloride as solvent. The shaded area indicates the distribution of the CRP fragments, which are shown fractionated with a mixture of standard proteins indicated by: Cat, equine hepatic catalase; CA, carbonic anhydrase; Lac, β-lactoglobulin; Lyz, lysozyme; Cyt *c*, horse heart cytochrome *c*; Cyt *c* [1–65], a CNBr fragment of cyt *c*; and InsA and InsB, bovine insulin A and B chains, respectively. Radioactivity was only found in association with CNBrI, indicating that both half-cystinyl residues of CRP are in this fragment.

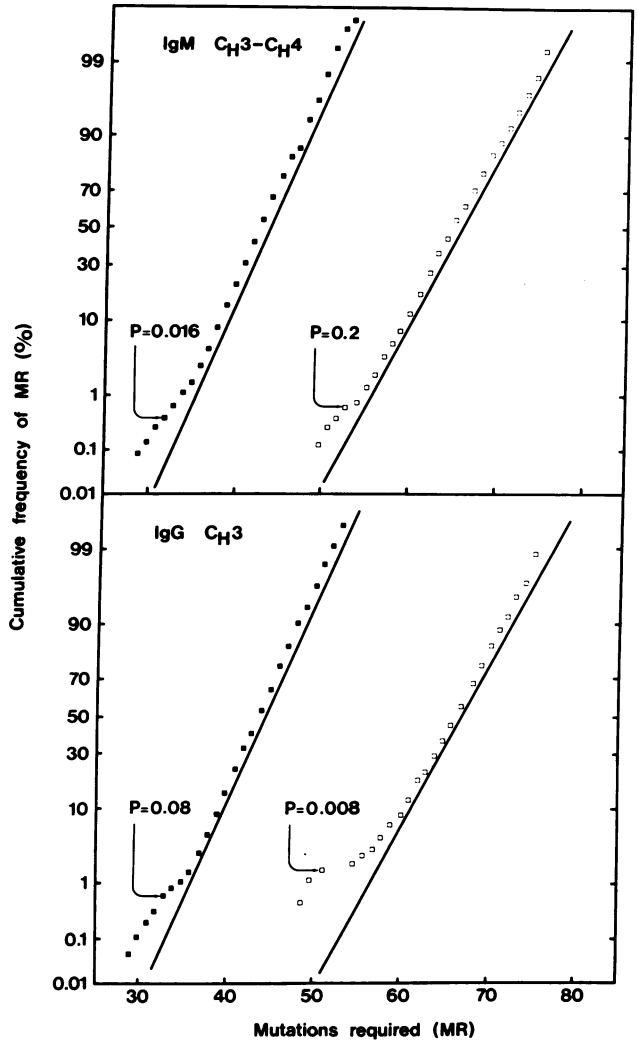

FIG. 3. Probability plots of cumulative frequencies of mutations required to interconvert lengths of sequence of 30 residues (■) and 45 residues (□) of rabbit CRP and (*upper*) C_H3-C_H4 domains of IgM (data from ref. 21) and (*lower*) C_H3 domain of IgG1 (data from ref. 20).

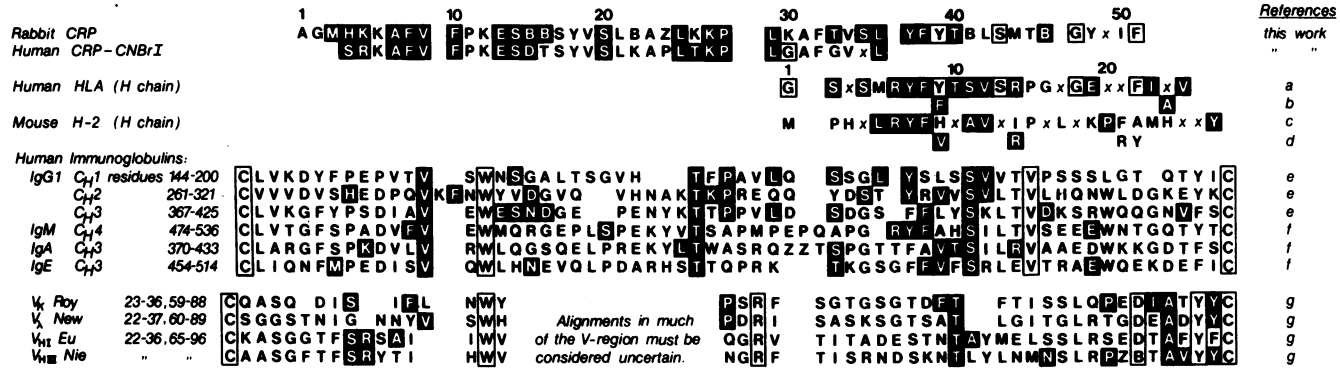

FIG. 4. Comparison of the amino-acid sequences of rabbit CRP, human CRP-CNBrI, histocompatibility antigens, and immunoglobulin constant (C) and variable (V) regions. Sequences are shown in the single letter notation, and shaded residues indicate the presence of identical residues in either a CRP or histocompatibility antigen sequence (or both) and at a comparable position in one or more of the immunoglobulin sequences presented. Where identical residues are seen between CRP and histocompatibility antigens, these are enclosed in boxes. The alignment of immunoglobulin C regions is adapted from refs. 20 and 21, and constant residues at any position are also enclosed in boxes. Alignment of immunoglobulin V regions, insofar as such alignment could be made, is adapted from ref. 19, and the residues enclosed are those reported to be constant in V regions (19). Hypervariable regions are not distinguished. The alignment between CRP and immunoglobulins is essentially that suggested by the Fitch analysis shown in Fig. 3, except that single gaps have been introduced in CRP between residues 9 and 10, 28 and 29, and 36 and 37 to maximize homology between CRP and immunoglobulin, and between residues 46 and 47 to improve homology between CRP and HLA "H" chain. The alignment between CRP and the histocompatibility antigens was suggested by the sequence Tyr-Phe-Tyr-Thr seen in human HLA-B7 (residues 7–10) and rabbit CRP (residues 37–40). Sources of sequence data are as follows: (*a*) human histocompatibility antigen HLA-B7 H chain (23, 28); (*b*) indicates the two residues where HLA-A2 H chain has been found to differ from HLA-B7 (23); (*c*) mouse histocompatibility antigen H-2K^k H chain (24–26); (*d*) indicates alternate residues seen in other H-2K or H-2D alloantigen sequences (24–27); (*e*) the sequence alignment of the C_H domains of IgG1 is adapted from Peterson *et al.* (20); (*f*) the sequence and alignment of the domains of IgM(C_H4), IgA(C_H3), and IgE(C_H4) homologous to the C_H3 domain of IgG1 are from Low *et al.* (21); (*g*) data for the V regions of immunoglobulins were arbitrarily selected from ref. 19.

0.016) was detected when 30, but not 45, residue lengths of sequence were compared.

The curves in Fig. 3, including the regions arising from nonhomologous pairing, show an unusual shift of about 0.8 nucleotide replacements to the left (*lower*). This indicates that another kind of nonrandomness is present. Its exact nature is not clear, but it could indicate that within immunoglobulins there is a pattern of weak internal sequence homology that is essentially reproduced in the CRP sequence. This effect is small, however, when compared to the deviations from random due to the comparison of homologous segments of CRP and the C_H3 domain (Fig. 3). The sequence alignment of CRP and immunoglobulins shown in Fig. 4 is based primarily on the region of homology indicated by these comparisons. The additional alignment with other domains of IgG1 (20) and IgM, IgA, and IgE (21) is justified by their established evolutionary interrelationships.

Homology of Histocompatibility Antigens with CRP and with Immunoglobulins. The unusual sequence in CRP, -Tyr-Phe-Tyr-Thr- (residues 37–40) was not found in any of the protein sequences currently listed in ref. 22, but it appears in the partial sequence of "heavy chains" from the human histocompatibility antigens HLA-7,12 recently reported by Terhorst *et al.* (23). This unexpected identity led us to compare the available sequence data for human and murine histocompatibility antigens (23–28) with the data for CRP. A total of 8 out of the 15 residues comparable in this alignment (Fig. 4) were identical. A comparison of the sequences of CRP, residues 30–51, and human HLA H chain, residues 1–22, by the diagonal match method of Gibbs and McIntyre (29) showed a highly significant ($P = 0.003$) relatedness between these sequences.

DISCUSSION

The determination of the primary structure of C-reactive protein and an evaluation of the evolutionary relationships of this protein to other proteins of the immune system are of significance in understanding their common and distinctive biological functions. Previous studies have demonstrated that the structure of CRP is distinct from that of immunoglobulin and other plasma proteins (2, 30), and the amino-acid sequence data presented here (Fig. 1) support this concept. However, the absence of the characteristic peptide chain structure of immunoglobulin does not rule out a distant evolutionary relatedness. Indeed, this was clearly established by the sequence comparison (Fig. 3), which indicated the alignment presented in Fig. 4. In addition, the homology between CRP and histocompatibility antigens indicated a region of homology between the latter proteins and immunoglobulins. The separate homologies noted between histocompatibility antigens and immunoglobulins in this comparison confirm the evolutionary interrelationships of these proteins.

When aligned according to Fig. 4, the NH_2-terminal residue of CRP corresponds to position 29 and the NH_2-terminal residue of the histocompatibility antigen corresponds to position 74 in the immunoglobulin V domain (numbering as in ref. 19). Thus, neither CRP nor histocompatibility antigen H chains possess the disulfide loop characteristic of an immunoglobulin domain, at least in the homologous NH_2-terminal segments of sequence presented here. Furthermore, there is no uniform conservation of the residues that could be considered essential for the maintenance of immunoglobulin domain structure that is indicated in Fig. 4. This is in contrast to the homology reported between the histocompatibility antigen light chain, β_2-microglobulin, and IgG1 (20, 31), where, with no exception, every residue that was conserved in C_L, C_H1, C_H2, and C_H3 was found in the homologous position in β_2-microglobulin (20). However, as is the case with CRP, the greatest homology was found with the C_H3 domain of IgG1. This probably reflects the conservative evolution of this region of IgG, imposed by the preservation of the primitive function of cell membrane association, a property shared by β_2-microglobulin.

The homologies that we have detected can be summarized as follows: for the simultaneous comparison of CRP with the immunoglobulin domains there are 24 identities in 50 positions

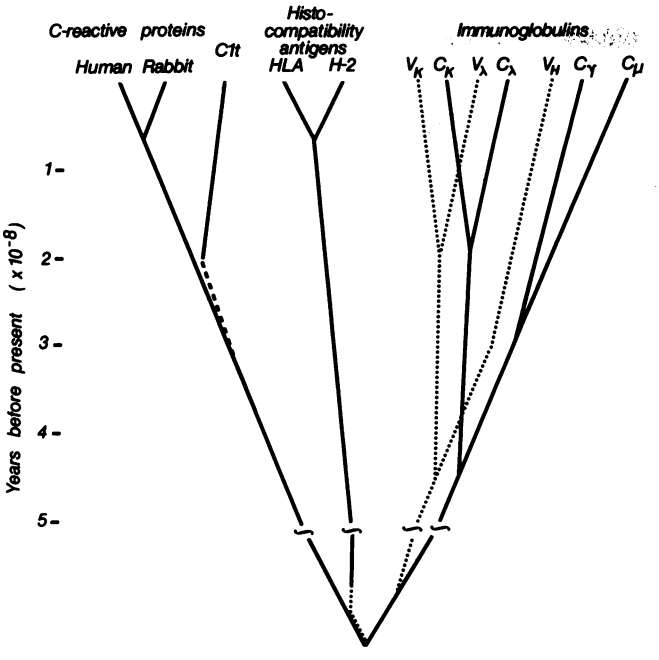

FIG. 5. Tentative scheme for the evolutionary origins of CRP, histocompatibility antigens, and immunoglobulins. The divergence of CRP and histocompatibility antigen is superimposed on the evolutionary tree of human immunoglobulins presented in ref. 19. The divergence of human and rabbit, and human and mouse, ancestral lines is assumed to have occurred about 75×10^6 years before the present. As presented elsewhere (33), the different extents of homology between human C1t and human CRP and rabbit CRP do not allow for confident estimation of this evolutionary divergence, which is placed somewhere between 200×10^6 and 300×10^6 years ago.

(48%), with the average identity with C_H domains being 16% and with V domains being 12%. Similarly, between the human HLA heavy chain and immunoglobulin C_H and V domains average identities of 21 and 14%, respectively, were calculated. Of 17 residue positions compared between CRP and human HLA-B7, 8 are identical (47%), but only 3 residues (21%) of 14 compared are identical with mouse H-2K^k sequence. Indeed, the composite sequences of human and mouse histocompatibility antigens presented here only show 33% identity, 5 residues out of 15 compared (for discussion and recent data for mouse H-2K^k, see *Note Added in Proof*). Of the sequences presented in Fig. 4 it would thus seem that CRP is most closely related to the human HLA heavy chain. In addition, as described elsewhere, human and rabbit CRP show about 58 and 43% identity, respectively, with the C1t subcomponent (32) of the complement component C1 (33). These structural relationships allow us tentatively to propose the evolutionary tree shown in Fig. 5. CRP, C1t, histocompatibility antigens, and the immunoglobulins have all developed from a common evolutionary precursor. The concept that the latter two groups of proteins share such an origin was initially proposed by Burnet (34). As evidenced by their relatively high homology, CRP and the heavy chains of histocompatibility antigens may have diverged more recently from one another than they did from the immunoglobulins. The similarity between C1t and CRP (33) indicates that their divergence was relatively recent in vertebrate evolution. Based on the observations that the levels of several complement components are controlled by genes closely linked to the major histocompatibility complex of several species (35), it has been suggested that certain complement components may be structurally homologous to the major histocompatibility antigens (36). This would seem to be true for the complement component C1t.

The functional relationships of the groups of proteins presented in Fig. 5 are less certain than their structural homologies. Immunoglobulins are generally accepted as being a diverse group of proteins that act as effector molecules in the recognition of foreign or "non-self" substances and in the subsequent activation of a variety of physiological reactions, either as humoral factors or in association with the membranes of various cells. Conversely, it has been suggested that the histocompatibility antigens are a highly polymorphic group of proteins that function primarily as recognition factors of "self" structures during differentiation (37). CRP has been found to interact with a variety of natural and synthetic macromolecules leading to the activation of the complement system (5–7). These include the pneumococcal C-polysaccharide, lecithin, and several naturally occurring and synthetic polycations, with no evident discrimination between autologously or heterologously derived substances. Hence CRP may be considered as a recognition factor both for "altered self" and "non-self" structures. From the ability of CRP to activate the complement system in a manner analogous to antibody, it might be expected that the homology with immunoglobulins would be with the C_H2 domain of IgG or C_H3 domain of IgM, both of which have been shown to contain the complement-fixing site of their respective classes. The absence of such a distinctive homology is not surprising because previous studies have failed to find a conservative sequence within these domains of immunoglobulins to which the function of complement fixation could be attributed (21). It would thus seem that this property is one of a topographic distribution of one or more reactive sites rather than one of a contiguous sequence of certain residues.

Independent of the ability of CRP to promote phagocytosis through complement-dependent opsonization, a direct stimulation of phagocytosis has been reported for CRP (4, 8). It is of interest to note that the sequence Thr-Lys-Pro at residues 26–28 in human CRP is directly homologous with that region in the C_H2 domain of IgG from which the phagocytosis-stimulating peptide, tuftsin, Thr-Lys-Pro-Arg, is derived (38). The sequence Thr-Lys-Pro was also found in the COOH-terminal peptide, CNBrII (Osmand and Friedenson, unpublished data).

The homology of CRP with the HLA histocompatibility antigen raises the question as to whether additional or stronger homology may be present between CRP and other gene products of the major histocompatibility complex, which presumably are homologous with the HLA antigens. The importance of these gene products in the regulation of the immune system has been well established (reviewed in ref. 35). The presence of an extensive homology between CRP and such proteins would suggest that CRP may possess immunoregulatory functions. Indeed the selective modulation of a variety of thymus-dependent lymphocyte properties has been reported for CRP (39, 40), and this protein may play a critical role in the control of lymphocyte functions during acute inflammatory reactions.

Note Added in Proof. It has been noted (see ref. 41) that at the positions in which assignments were not made in the sequence of mouse histocompatibility antigen (Fig. 4) the human histocompatibility antigen sequences contained a residue that had not been tested. The presence of identical residues at these positions would lead to a considerable increase both in the homology between HLA and H-2K^k heavy chains and, to a lesser extent, in the homology between CRP and H-2K^k heavy chain. Preliminary evidence supports this contention, and indicates

that the extent of the homology between HLA and H-2K^k will be greater than that between either CRP and HLA or CRP and H-2K^k. The homologies cited in the *text* reflect the nature of the information upon which the figures are based rather than the evolutionary interrelationships proposed in Fig. 5.

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